method that provides an estimate of the standard deviation. The protective unit value per total human immunizing dose of the vaccine under test shall be calculated in terms of the unit value of the standard vaccine.

(g) *Potency requirements.* The vaccine shall have a potency of 12 units per total human immunizing dose based upon either a single test estimate of no less than 8 units or a two-, three- or four-test geometric mean estimate of no less than 9.6, 10.8, or 12 units, respectively, except that for the vaccine in a multiple antigen product containing Poliovirus Vaccine Inactivated, the estimate shall be no less than 14 units. In no event shall the estimate be more than 36 units.

(h) *Test design variation.* Variations in the design of the potency test may be permitted providing the results are demonstrated to be of equal or greater precision.

[38 FR 32064, Nov. 20, 1973, as amended at 50 FR 4137, Jan. 29, 1985]

§ 620.5 Mouse toxicity test.

The final vaccine shall be demonstrated to be free from toxicity by the following test:

A group of no less than 10 mice, each mouse weighing 14 to 16 grams, shall have free access to food and water for no less than 2 hours before injection. The group weight of the mice shall be determined immediately prior to injection. Each mouse shall be injected intraperitoneally with a test dose of one-half of the largest recommended single human dose of the final vaccine in a volume of no less than 0.5 ml. nor more than 0.75 ml. The group weight of the mice shall be determined at the end of 72 hours and at the end of 7 days after injection. At the end of 72 hours the average weight per mouse may be no less than the average weight per mouse immediately preceding the injection; at the end of 7 days the average weight gain per mouse may be no less than 3.0 grams; and at the end of 7 days there may be vaccine-related deaths of no more than 5 percent of the total number of mice in all the toxicity tests performed.

§ 620.6 General requirements.

(a) *Safety.* Each lot of product containing Pertussis Vaccine shall be tested for safety by the procedures prescribed in § 610.11 of this chapter except that the test shall consist of the intraperitoneal injection of no less than one-half of the recommended largest individual human dose into each of the mice, and either the intraperitoneal injection of no less than three times the recommended largest individual human dose, or the subcutaneous injection of 5.0 milliliters into each of the guinea pigs.

(b) *Dose.* These additional standards are based on a single injection of 0.5 ml., 1.0 ml., or 1.5 ml., and a total human immunizing dose of three single injections of a nonadsorbed vaccine, and two or three single injections of an adsorbed vaccine.

(c) *Product characteristics.* Recommendations shall be made through appropriate labeling that the product after issue should not be frozen and should be well shaken immediately prior to use.

(d) *Labeling.* In addition to the items required by other applicable labeling provisions of this part, the package label shall give the following information:

(1) For a vaccine containing a precipitant or an adsorbent, the word ''Adsorbed'' shall follow the proper name in the same style of type and prominence as the proper name.

(2) The total immunizing dose contains 12 units of pertussis vaccine.

(e) *Multiple antigen products.* The Pertussis Vaccine components of multiple antigen products shall be manufactured pursuant to these additional standards, except that the mouse toxicity test (§ 620.5) and the potency test (§ 620.4) shall be performed on the multiple antigen product.

(f) *Adsorbed vaccines.* Only aluminum compound reagents shall be introduced into the product to cause precipitation or adsorption of either Pertussis Vaccine or other antigens incorporated with Pertussis Vaccine.

(g) *Freezing prohibition.* Pertussis Vaccine and multiple antigen products of which Pertussis Vaccine is a component shall not be frozen at any time during storage.

(h) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(1) A sample of no less than 20 milliliters of the final product for pertussis vaccine testing.

(2) Protocols showing summaries of the manufacturing processes and the results of all mouse toxicity (§ 620.5) and potency (§ 620.4) tests performed.

[38 FR 32064, Nov. 20, 1973, as amended at 41 FR 35480, Aug. 23, 1976; 48 FR 13025, Mar. 29, 1983; 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart B—Typhoid Vaccine

§ 620.10 Typhoid Vaccine.

The proper name of this product shall be Typhoid Vaccine which shall be an aqueous or dried preparation of killed *Salmonella typhi* bacteria.

[48 FR 7167, Feb. 18, 1983]

§ 620.11 Production.

(a) *Strain of bacteria.* (1) Strain Ty 2 of *Salmonella typhi* shall be used in the manufacture of Typhoid Vaccine.

(2) The antigenic integrity of the Ty 2 strain shall be verified by an appropriate serological procedure.

(b) *Propagation of bacteria.* The culture medium for propagation of *S. typhi* shall not contain ingredients known to be capable of producing allergenic effects in human subjects. The harvested bacteria shall be free of extraneous bacteria, fungi, and yeasts, as demonstrated by microscopic examination and cultural methods.

(c) *Bacterial content.* (1) The number of bacteria in the concentrate of harvested bacteria shall be estimated not later than 2 weeks after harvest and before any treatment capable of altering the accuracy of the estimate.

(2) The number of *S. typhi* bacteria in the vaccine shall not exceed 10^9 per milliliter.

(d) *Nitrogen content.* The total nitrogen content of the vaccine shall not exceed 0.035 mg./ml. for nonextracted bacteria preparations and shall not exceed 0.023 mg./ml. for acetone-extracted bacteria preparations.

(e) *Preservative.* Aqueous vaccine and the solution for reconstitution supplied with dried vaccine shall contain a preservative. Dried vaccine shall not contain a preservative.

[38 FR 32064, Nov. 20, 1973, as amended at 48 FR 7167, Feb. 18, 1983]

§ 620.12 U.S. Standard preparations.

The following U.S. Standard preparations shall be obtained from the Center for Biologics Evaluation and Research (HFB–210), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, for use as prescribed in this part:

(a) *Vaccine standard.* The U.S. Standard Typhoid Vaccine for determining the potency of Typhoid Vaccine.

(b) *Opacity standard.* The U.S. Opacity Standard for adjusting the opacity of the suspension from which the challenge culture is prepared.

[48 FR 7167, Feb. 18, 1983, as amended at 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11015, Mar. 26, 1990]

§ 620.13 Potency test.

The number of potency units per milliliter shall be estimated for each lot of vaccine from the results of simultaneous mouse protection tests of the vaccine under test and of the U.S. Standard Typhoid Vaccine. At least four dilutions of each lot of vaccine shall be tested. The test shall be performed as follows:

(a) *Mice.* Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weights between 13 and 16 grams. A system of randomization shall be used to distribute the mice into the groups, with respect to shelf position and to determine the order of challenge. A group of at least 16 mice shall be used for each dilution of each vaccine. There shall be at least 4 groups consisting of no less than 10 mice each for control testing purposes, as required under paragraph (c) of this section.

(b) *Inoculation of vaccine.* (1) Serial dilutions, no greater than fivefold, of the vaccine to be tested and of the standard vaccine shall be made in saline (0.85 percent sodium chloride solution or phosphate-buffered saline). The mean